Supplemental Data

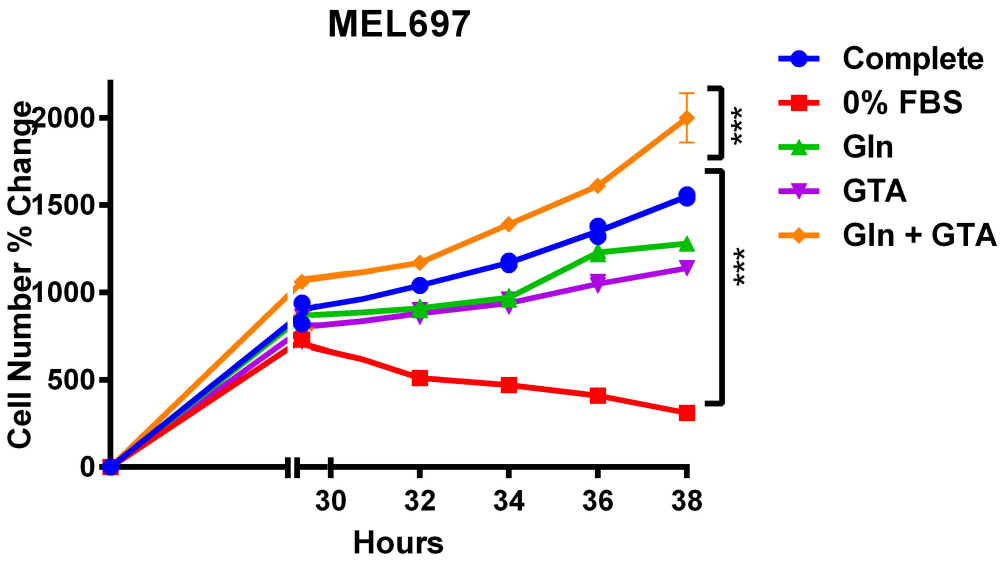

Fig. S1. MEL697 cells were seeded in a 24 well plate at density of 2 x 10^4 cells per well in regular cell culture medium and incubated for 12 hours. Regular medium was replaced with conditioned medium containing indicated nutrients and cells were cultured for 30 hours. Cell counting was performed at indicated time points and percent cell number change plotted. ***<0.001

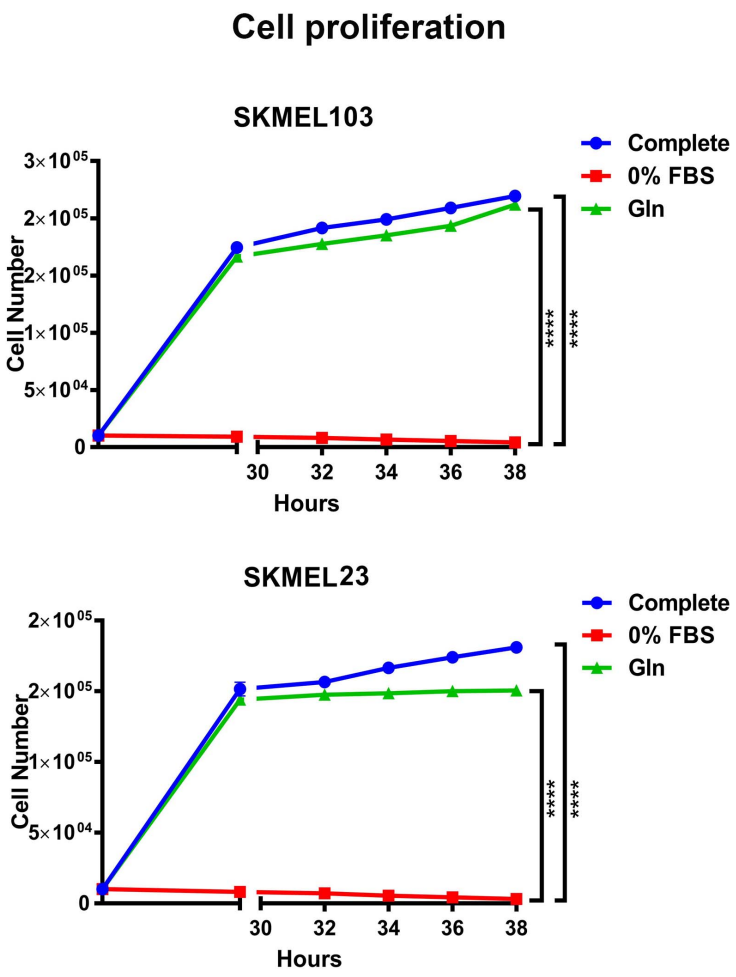

Fig. S2. SKMEL103 or SKMEL23 cells were seeded in regular cell culture medium and incubated for 12 hours. Regular medium was replaced with conditioned medium containing indicated nutrients and cells were cultured for 38 hours. Cell counting was performed at indicated time points and cell number change plotted. ***<0.001

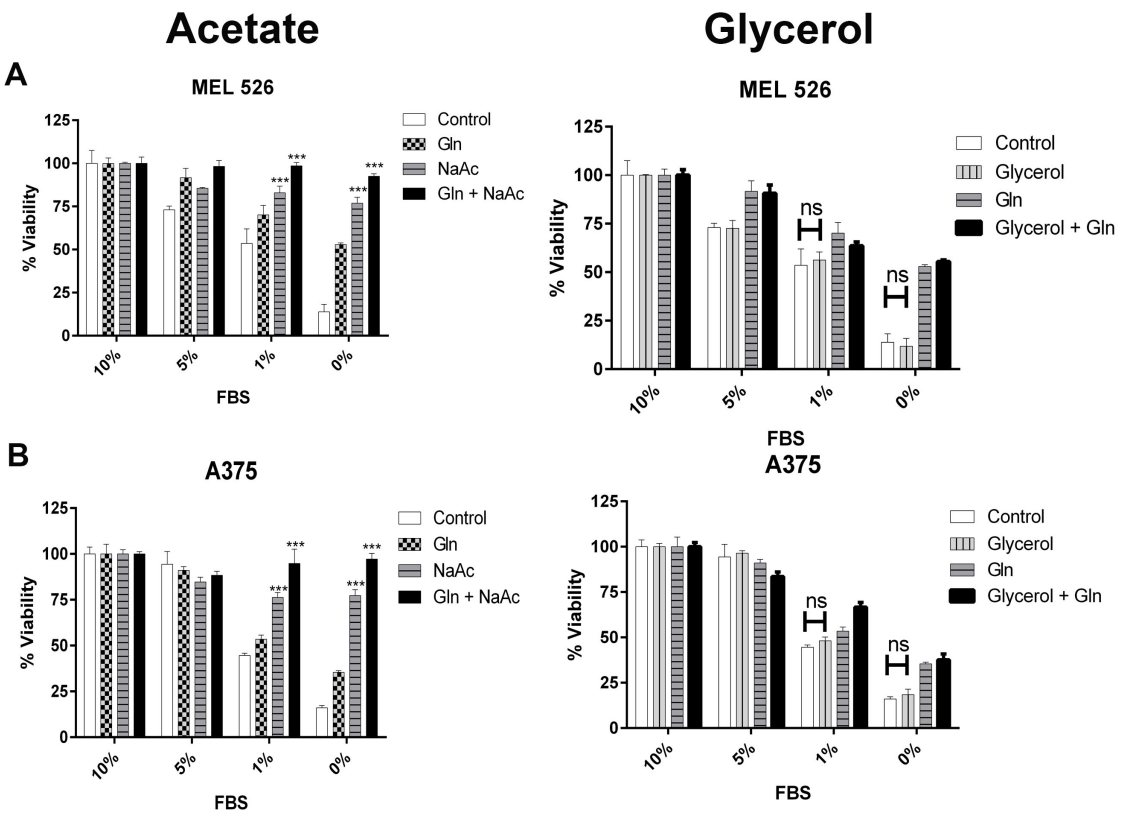

Fig. S3. Cell viability of MEL526 (A) and cells A375 (B) grown in defined medium with added nutrients as indicated. Viability was assessed using a modified Alamar Blue reagent by fluorometry. Values represent averages of triplicates. Statistical analysis was conducted using Bonferroni's Multiple Comparison Test; ***P<0.001, ns: not significant.

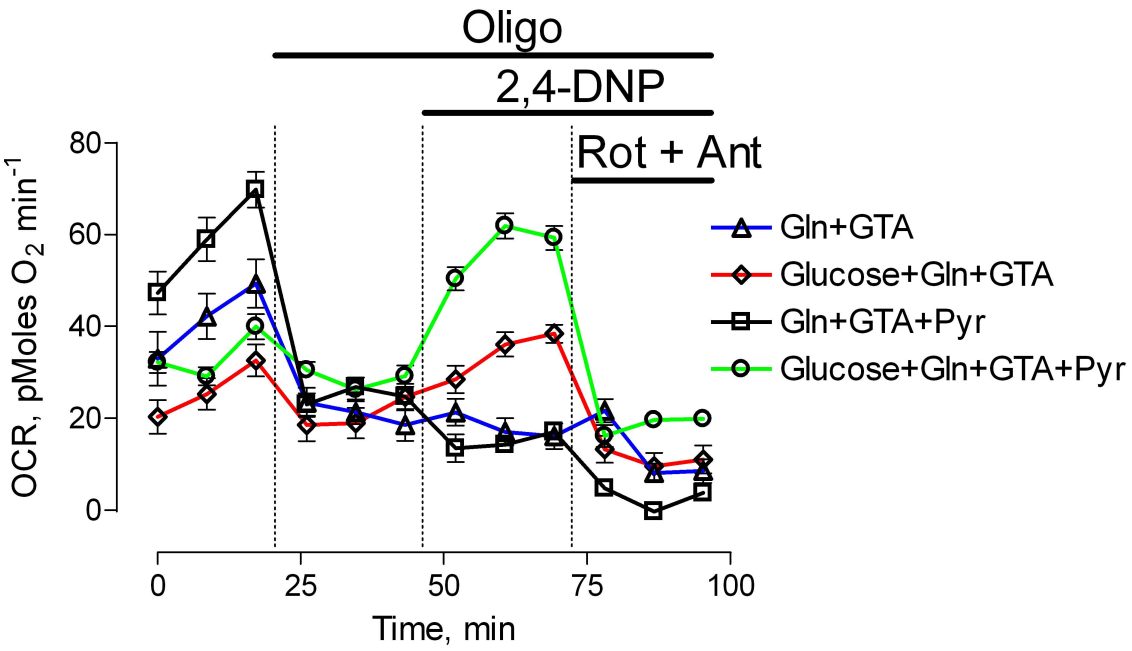

Fig. S4. OCR measurements of glucose replete or deplete melanoma cells in the presence or absence of methyl-pyruvate. Each data point is an average of five replicates.

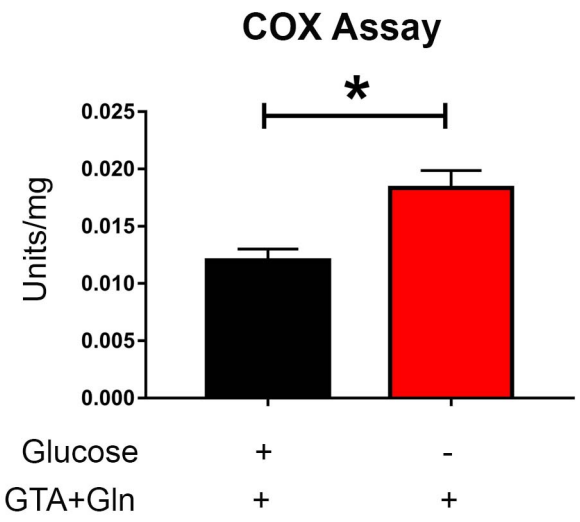

Fig. S5. Mitochondria isolated from glucose-replete or –deplete melanoma cells and COX activity assessed. $p<0.05$

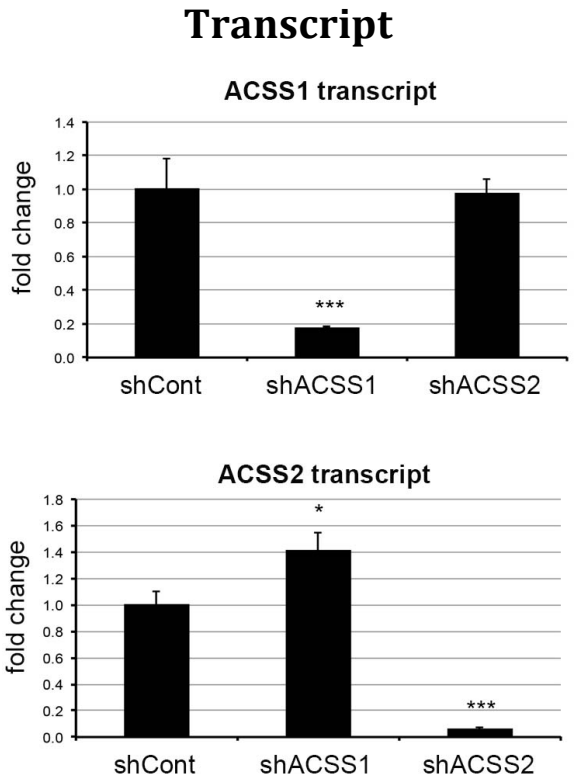

Fig. S6. Transcript: ACSS1 and ACSS2 transcript levels in MEL 526 cells lentivirally transduced with shRNA corresponding to respective target genes. *P<0.05, ***P<0.001. Protein: Cell extracts prepared from ACSS1/2 knockdown cells were blotted with indicated antibodies.

Supplemental method:

Melanoma cells were cultured in glucose-replete or –deplete medium. Mitochondria were isolated from these cells using mitochondria isolation kit (Thermo Scientific, Cat# 89874). Cytochrome c Oxidase activity of these mitochondrial fractions were assessed using Cytochrome c Oxidase Assay Kit from Sigma (Cat# CYTOCOX1).